Journal of Andrology, Vol. 28, No. 1, January/February 2007

Ejaculate Volume Is Seriously Underestimated When Semen Is Pipetted or Decanted Into Cylinders From the Collection Vessel

Andrology Lab Corner*

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The total sperm count (number of spermatozoa per ejaculate) rather than sperm concentration (number of spermatozoa per unit volume of semen) is the more important semen variable related to fertility. It reflects testicular volume (Handelsman et al, 1984; Andersen et al, 2000; Behre et al, 2000), and thus is a measure of total testicular sperm output (MacLeod and Wang, 1979), which is directly related to the chances of pregnancy after coitus. The concentration of spermatozoa in the ejaculate, however, depends on the extent of dilution of epididymal spermatozoa by secretions of the prostate and seminal vesicles occurring at ejaculation and is therefore influenced by the secretory capacity of the accessory sex glands. This is an important distinction, for when comparing semen quality from older and younger men, sperm concentrations do not differ, yet semen volume is reduced in the older men, and so the total number of spermatozoa per ejaculate is lower in the older men (Ng et al, 2004; Nieschlag et al, 1982). The total number of spermatozoa per ejaculate is obtained by multiplying the concentration of spermatozoa by the semen volume. The latter is best measured by weighing (Eliasson, 2003), assuming a density of 1.0 g/mL (Auger et al, 1995; Jorgensen et al, 1997, 2001; Brazil et al, 2004), but alternative methods, such as collection into graduated cylinders (Behre et al, 2000), pipetting from the collection vessel (Mortimer 1994; Jorgensen et al, 1997), and pouring from the collection vessel into a graduated tube (Jorgensen et al, 1997), are in current practice.

Two recent studies have found that pipetting semen from the collection vessel leads to an underestimation of about 0.5 mL (range 0.3–0.8 mL; Brazil et al, 2004; Iwamoto et al, 2006) compared with weighing, but no data are available about losses incurred when pouring semen into graduated cylinders. Because the area of contact with the sides of the collection vessel while decanting semen into a graduated cylinder is likely to be far larger than that during pipetting, retention within the vessel could be much larger, leading to a larger underestimation of volume with this method. In this study, new data are obtained on the loss of semen volume during decanting to a cylinder and previously published results on losses because of pipetting, and the density of semen is reanalyzed together with additional data.

Methods and Validation of Equipment

Semen—Nine healthy donors provided semen by masturbation at the University of California, Davis, laboratory after at least 2 days of sexual abstinence. Additionally, data from previous publications from the Study for Future Families (SFF), a multicenter study of semen quality from fertile men in the United States (Brazil et al, 2004) were reanalyzed along with data from nearly 300 additional men from the same study but which were collected subsequent to the published

This work was supported by grants from the National Institutes of Health (R01-ES09916 to the University of Missouri, MO1-RR00400 to the University of Minnesota, MO1-RR0425 to Harbor-UCLA Medical Center) and by grant 18018278 from the State of Iowa to the University of Iowa.

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Received for publication July 26, 2006; accepted for publication September 6, 2006.

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DOI: 10.2164/jandrol.106.001297

analyses for the volume vs pipetting comparisons. A subset of 80 men was used to collect the data on the weight of 1.0 mL of semen.

Statistics—All statistical tests were performed with Sigma Stat (Erkrath, Germany) and statistical significance was accepted at P less than 0.05. The use of parametric or nonparametric tests was dictated by the nature of the untransformed data, and the relevant tests used are mentioned in the text. Unless otherwise noted, data are presented as mean ± SD, range.

Accuracy of Equipment—The balance (Denver Instruments, Arvada, Colo) was accurate up to 210 g, and internal calibration was invoked when it was switched on. Standards of 1, 2, 5, and 10 g were weighed 10 times, each time after switching on the balance. The mean weights were 0.999, 2.000, 4.999, and 9.999 g, with coefficients of variation 0.042%, 0.021%, 0.010%, and 0.004%, respectively. The mean accuracy of the balance was 99.9%.

The weight of 35, 120-mL semen collection vessels was 17.261 ± 0.667 g (range 15.380–18.270 g, CV 3.8%), emphasizing the need to weigh each empty collection vessel individually and not assume a standard weight for the container.

Plastic 5.0-mL pipettes (Fisher, Pittsburgh, Pa), with 0.1-mL graduations, were calibrated 2 ways: first, by aspirating water to the 3.0-mL line and then expelling it into tared weighing boats and, second, by aspirating into the pipettes exactly 3 g of water and then reading the volume from the pipette scale. Each procedure was repeated 10 times. The weight of exactly 3.0 ml of water, as measured by the pipette (2.989 ± 0.035 g, 2.935–3.057 g), was 99.7% of the anticipated weight of that volume of water (2.995) from its density (0.9982 g/mL at ambient temperature of 20°C; Lentner 1981). Three grams of water (2.999 ± 0.006 g, 2.990–3.010 g) was measured to a volume of 3.0 ± 0.0 (3.0–3.1) mL, which was 96.5% of the volume anticipated from its density (3.004 ±0.006 mL, 2.955–3.015 mL).

Positive-displacement pipettes (Microman M-1000, Oakland, Calif), were calibrated by expelling different volumes of water into tared weighing boats in 4 SFF centers. As part of regular quality control, the weights of exactly 100, 950, 1000, and 1900 µL of water (99.555 ±1.372, 93.9–106.0; 954.613 ± 3.958, 934.5–953.3; 0.992 ± 0.005, 0.977–1.003; and 1893.613 ± 3.771, 1886.0–1901.0 mg, respectively) represented a mean recovery of 99.6% of the anticipated weight of that volume of water calculated from its density.

Glass 10.0-mL measuring cylinders (Pyrex, Acton, Mass), with 0.1-mL graduations, were calibrated by 2 methods. In the first, 3 g of water (dispensed into weighing boats) was transferred to cylinders, and the volume was read off the cylinder scale. No loss of water was observed during transfer. In the second method, exactly 3.0 mL of water, as determined from the cylinder graduations, was added to preweighed cylinders that were then reweighed. Each procedure was repeated 10 times. The anticipated volume of this weight of 3 g of water, calculated from the weight transferred (3.013 ± 0.013, 2.990–3.030 mL) and either assuming a density of water of 1.00 g/mL or employing 0.9982 g/mL (3.018 ± 0.013, 2.995–3.0335 mL), was 99.4% of the measured volume (3.0 ± 0.1, 2.9–3.1 mL). The weight of 3.0 mL of water (2.976 ± 0.055, 2.880–3.080 g) was 99.6% of the weight anticipated from its density (2.965 g).

Experiments and Results

Comparison of Weighing and Pipetting on Semen Volume—Semen samples from 803 SSF men (1 or 2 per man) were collected directly into disposable collection vessels that had been previously weighed. The empty container contained a label with the subject's information on which the vessel's weight was recorded. The vessel was capped during liquefaction at room temperature and was weighed again after liquefaction. Within 20 to 30 minutes of ejaculation, the specimen container was tipped to about 45° so that semen collected at the base/side at an angle to facilitate pipetting. Semen was aspirated into the pipette with a pipette pump (Fisher), with due care being taken to remove all the semen after waiting for it to accumulate in the angle of the container. Volume was estimated to the nearest 0.1 mL.

The volume of 1429 semen samples from 803 men in 4 US centers was calculated from the sample weights. Whether the density of semen was assumed to be 1.0 g/mL (3.888 ± 1.682, 0.120–11.470 mL), 0.9882 g/mL (3.895 ± 1.685, 0.120–11.491 mL), or 1.014 g/mL (the density of semen estimated below: 3.834 ± 1.659, 0.118–11.312 mL), the volume by weight was significantly greater (Wilcoxon signed rank test) than that measured by pipette (3.4 ± 1.6, 0.1–10.6 mL). The mean difference (assuming a density of 1 g/mL) was 0.500 ± 0.266 mL (−0.600–2.890 mL). Only 5 samples (0.3%) were measured to have larger volumes by pipetting than weighing, and only 3 samples (0.2%) had volumes from weighing exceeding that by pipetting by more than 2.0 mL. The extent of the loss represented 14.3 ± 8.3% (−20.0%–75.0%), with the larger percentage errors associated with smaller semen volumes.

Comparison of Weighing and Decanting Into a Cylinder on Semen Volume—Two experiments were performed: the first to mimic normal laboratory handling of vessels and the second with a more careful handling protocol in which semen was poured directly into the bottom of a specimen container designed to minimize contact of semen with the sides of the collection vessel before measurement. For normal laboratory handling, semen

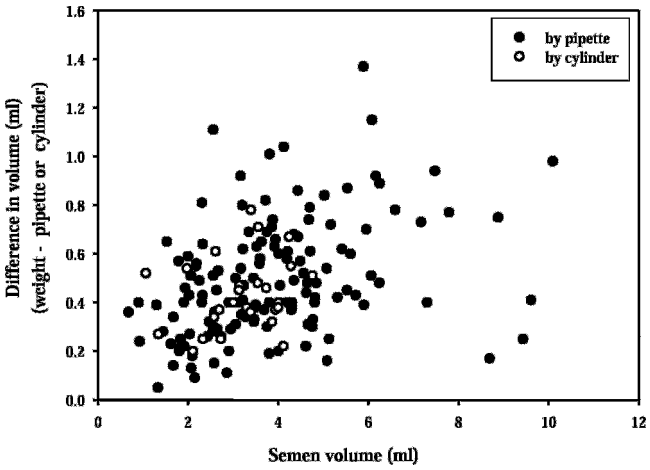
was collected directly into preweighed disposable collection vessels as above. After liquefaction and weighing, the semen was poured into a graduated cylinder. Adequate time was given to allow the semen to drain from the vessel into the cylinder (10 seconds), and the last drops were collected by tapping the collection vessel against the lip of the cylinder. Additional time was allowed for semen to drain from the walls of the cylinder before the volume reading was taken. The volume was read from the meniscus to the nearest 0.1 mL. After removing the maximum amount of semen in this way, the collection vessel was reweighed to ascertain the amount of residual semen remaining in the vessel after decanting its contents.

The volume of 44 semen samples, calculated from the weights assuming a density of 1.0 g/mL (3.108 ± 0.977, 1.200–5.010 g), 0.9982 g/mL (3.113 ± 0.979, 1.202–5.019 g), or 1.014 g/mL (3.065 ± 0.963, 1.1783–4.941 g) was significantly greater (paired *t* test) than that measured from the graduated cylinder (2.7 ± 0.9, 0.8–4.5 mL). The mean difference (assuming a density of 1 g/mL) was 0.377 ± 0.150 (0.100–0.78) mL. The extent of the loss was in the range 3.3%–39.4% (12.9 ± 6.3%) of the volume estimated by weighing. This estimate of the loss, derived from comparing the measured volumes and weights, was significantly larger (paired *t* test) than the extent of loss determined from the weight of residual semen in the collecting vessel after decanting (0.321 ±0.104, 0.130–0.600 mL).

Of the 27 samples in which routine laboratory conditions were employed, loss of semen was significantly more (*t* test; 0.427 ± 0.151, 0.200–0.780 mL) than when precautions were taken (n = 17) to avoid excess contact of semen with the sides of the vessel before decanting (0.297 ± 0.111, 0.10–0.490 mL). Residual semen loss (0.368 ± 0.099, 0.170–0.600 mL) was also greater than when more care was taken in handling the samples (0.242 ± 0.052, 0.130–0.350 mL).

Density of Semen—The density of semen was measured by weighing 1.0 mL, dispensed with a positive displacement pipette, into tared weighing boats. Eighty semen samples from 4 different SFF centers had a mean density of 1.014 ± 0.0133, 0.970–1.043 g/mL. Estimates between centers were not significantly different.

Comparison of Weighing, Pipetting, and Use of a Graduated Cylinder on Semen Volume—The volume of semen estimated by weighing the collection vessel before and after ejaculate collection, and assuming its density to be 1 g/mL, was consistently greater than that estimated by measuring its volume with a pipette or by pouring into a graduated cylinder. This is shown in the Figure, in which the difference between weights is plotted against the mean weight from the compared methods (Bland and Altman, 1995). The underestimate



The difference in volume of 166 semen samples estimated by weighing the collection vessel and that measured by aspiration into a pipette (●) or decanting into a graduated cylinder (○) plotted against the mean volumes from the two compared methods (abscissa). The cylinder data presented are only those for ejaculate volume collected directly into the collection vessel (n = 27) but only one tenth of the pipette data are shown for clarity (every tenth datum from the list ordered in increasing weight-volume difference, N = 138).

of semen volume by transferring the sample from the collection vessel to a cylinder (0.427 mL) was significantly lower (Mann-Whitney rank sum test) than the loss incurred by pipetting from the vessel (0.500 mL). For both methods, the actual underestimate of semen volume was positively related to the volume of the semen estimated by weighing, although when expressed as a percentage of the total volume, an increased loss with lower semen volume was found (data not shown).

Discussion

This study has shown that a consistent and significant reduction in the volume of semen is obtained when a pipette or a graduated cylinder is used to measure liquefied semen transferred from its collection vessel. These losses cannot be accounted for by evaporation because samples were capped during liquefaction at room temperature and pipetted or decanted immediately after weighing. It could be that with particularly viscous samples, transfer would result in even lower volumes because more would be retained on the side of the decanting vessel and some might adhere to the sides of the cylinder. The difference in estimates of semen volume by weighing and pipetting has been reported before (Brazil et al, 2004; Iwamoto et al, 2006) but only mean values were given. In this study, the loss of semen was similar (~0.5 mL) and represented a mean of 14% loss of volume. The new data on loss of semen associated with pouring into a graduated cylinder revealed a similar underestimation of semen volume

(~0.4 mL) that represented a similar percent loss of semen (13%).

Iwamoto et al (2006) used their measured mean difference (0.49 mL) to correct semen volumes to compare results with other studies in which weighing was used to estimate semen volume. Jorgensen et al (1997) reported laboratories that assumed 0.1 mL of semen was left after decanting into a graduated tube and added this value to the volume measured. The results of this study suggest such a correction procedure would introduce even greater errors because the range of loss varied considerably, perhaps related to the inherent viscosity of the sample or the handling of the sample after collection and, thus, argues against this practice.

The density of human semen has been published before (Huggins et al, 1942; Brazil et al, 2004), but again, only mean values were reported. Reanalysis of the data from Brazil et al (2004) and analysis of additional samples provided values that are somewhat lower than the mean reported by Huggins et al (1942), for which no details of the methodology were given. The density of water established by exactly the same method was close to that reported for water at 20°C (Lentner 1981), confirming the accuracy of the value. A factor of 1.00 is thus sufficient for purposes of estimating semen volume from its weight.

Semen volume is best measured by weighing the sample in the collection vessel (and assuming a density of 1 g/mL, which is very close to the measured value of 1.014 g/mL) rather than pipetting or decanting the semen into a graduated cylinder because this subsequent transfer to measuring devices brings underestimates of volume that will compromise accuracy of total spermatozoan counts or other cells in the ejaculate.

Acknowledgments

We thank Fan Liu, Cathy Treece, and Charlene Tollner (Center for Health and the Environment, University of California, Davis, California) for providing original data and technical support. The Study for Future Families Research Team includes: E.Z. Drobnis, B.S. Carter, D. Kelly, S.L. Stewart, and T.M. Simmons (University of Missouri-Columbia); C. Wang, L. Lumbreras, S. Villanueva, M. Diaz-Romero, M.B. Lomeli, R.S. Swerdloff, A. Victoroff, R. Sandoval, S. Bavarian, A. Leung, A.L. Nelson, and E. Otero-Salazar (Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center); J.B. Redmon, C. Kwong T. Perrier, T. Srb, J. Pryor, C. DeJonge, and A. Muehlen (University of Minnesota); A. Sparks, A. Wolf, J. Whitham, M. Hatterman-Zogg, M Slaughter, T. Grinder, and M. Maifeld (University of Iowa). We thank the health care providers and study participants at University Physicians Clinic (Columbia, Mo), Fairview Riverside Women's Clinic (Minneapolis, Minn), Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center (Los Angeles, Calif), Cedars-Sinai Medical Center (Los Angeles, Calif), and University of Iowa Hospitals and Clinics (Iowa City, Iowa).

References

Andersen AG, Jensen TK, Carlsen E, Jorgensen N, Andersson AM, Krarup T, Keiding N, Skakkebaek NE. High frequency of suboptimal semen quality in an unselected population of young men. *Hum Reprod*. 2000;15:366–372.

Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med*. 1995;332:281–285.

Behre HM, Yeuing CH, Holstein AF, Weinbauer GF, Gassner P, Nieschlag E. Diagnosis of male infertility and hypogonadism. In: Nieschlag E, Behre HM, eds. *Andrology, Male Reproductive Health and Dysfunction*. Berlin: Springer, 2000:92.

Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard measurement is misleading. *Lancet*. 1995;346:1085–1087.

Brazil C, Swan SH, Drobnis EZ, Liu F, Wang C, Redmon JB, Overstreet JW. Standardized methods for semen evaluation in a multicenter research study. *J Androl*. 2004;25:635–644.

Eliasson R. Basic semen analysis. In: Matson P, ed. *Current Topics in Andrology*. Perth: Ladybrook Publishing; 2003;35–89.

Handelsman DJ, Conway AJ, Boylan LM, Turtle JR. Testicular function in potential sperm donors: normal ranges and the effects of smoking and varicocele. *Int J Androl*. 1984;7:369–382.

Huggins C, Scott WW, Heinen JH. Chemical composition of human semen and of the secretions of the prostate and seminal vesicles. *Am J Physiol*. 1942;136:467–473.

Iwamoto T, Nozawa S, Yoshiike M, Hoshino T, Baba K, Matsushita T, Tanaka SN, Naka M, Skakkebaek NE, Jorgensen N. Semen quality of 324 fertile Japanese men. *Hum Reprod*. 2006;21:760–765.

Jorgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J, Petersen JH, Andersen AN, Auger J, Cawood EH, Horte A, Jensen TK, Jouannet P, Keiding N, Vierula M, Toppari J, Skakkebaek NE. Regional differences in semen quality in Europe. *Hum Reprod*. 2001;16:1012–1019.

Jorgensen N, Auger J, Giwercman A, Irvine DS, Jensen TK, Jouannet P, Keiding N, Le Bon C, MacDonald E, Pekuri AM, Scheike T, Simonsen M, Suominen J, Skakkeboek NE. Semen analysis performed by different laboratory teams: an intervariation study. *Int J Androl*. 1997;20:201–208.

Lentner C, ed. *Geigy Scientific Tables*. Vol 1, 8th ed. Basle, Switzerland: Ciba-Geigy Ltd; 1981;50.

MacLeod J, Wang Y. Male fertility potential in terms of semen quality: a review of the past, a study of the present. *Fertil Steril*. 1979;31:103–116.

Mortimer D. *Practical Laboratory Andrology*. New York, NY: Oxford University Press; 1994;49.

Ng KK, Donat R, Chan L, Lalak A, Di Pierro I, Handelsman DJ. Sperm output of older men. *Hum Reprod*. 2004;19:1811–1815.

Nieschlag E, Lammers U, Freischem CW, Langer K, Wickings EJ. Reproductive functions in young fathers and grandfathers. *J Clin Endocrinol Metab*. 1982;55:676–681.